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SPECIFICATION FOR FISH PROTEIN CONCENTRATE

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Indian Standard

SPECIFICATION FOR FISH PROTEIN CONCENTRATE

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(*Continued on page* 27)

Indian Standard
SPECIFICATION FOR FISH PROTEIN CONCENTRATE

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 31 December 1981 after the draft finalized by the Nutrition Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 The nutritive value of proteins from fish in many instances is the same as that of milk proteins. Fish proteins are fairly well-balanced in most of the essential amino acids, and are also generally rich in lysine; which is known to be deficient in most cereals, millets, root crops, etc, which form the bulk of the diet of more than two-thirds of the world population. It is now well established that supplementation of diets based on cereals and such other products with fish even at low levels greatly improves the growth-promoting value of the former.

0.3 Increasing attention has been paid during recent years to the conversion of fish material into an edible quality of fish flour or fish protein concentrate conforming to prescribed nutritional and hygienic standards. Fish protein concentrate is a colourless, odourless, tasteless, blanel and dry powder prepared from fish by a hygienic solvent extraction process for human consumption. It contains a high percentage of protein and less than 0·75 percent of fat.

0.3.1 The formulation of this Indian Standard is expected to help in exercising proper quality control in fish protein concentrate prepared particularly from that variety of fish which may not otherwise be consumed.

0.4 A recommended method for the preparation of fish protein concentrate is given in Appendix K.

0.5 In the preparation of this standard, due consideration has been given to the relevant Rules laid down under the Prevention of Food Adulteration Act, 1954 and the standards of Weights and Measures (Packaged Commodities) Rules, 1977. The standard is, however, subject to the restrictions imposed under these, wherever applicable.

0.6 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and test for fish protein concentrate.

2. RAW MATERIALS

2.1 The fish used shall itself be in a condition fit for human consumption.

2.2 The fish used may preferably be inexpensive varieties, for example, silver belly (*Leiognathus* sp), *dhoma*, jew fish (*Pseudorciaena* sp) or malabar sole (*Cyanolossus* sp) and shall be free from selenium, neurotoxins and other organo-metallic impurities (*see* Table 1).

2.3 The fish should be dressed and eviscerated to remove less desirable portions such as the intestines and then washed with water.

3. REQUIREMENTS

3.1 Description — Fish protein concentrate shall be in the form of a fine colourless powder that is easy to blend and shall not have more than a faint odour and taste when added to boiling water in a closed container. It shall be prepared by a hygienic solvent extraction process. It shall be free from dirt, or other extraneous matter, added colouring and flavouring agents.

3.2 Any of the following solvents shall be used in its preparation:

a) *Ethanol* — 96 percent (*v/v*) (food grade),
b) *Iso-propanol* — food grade, and
c) *Hexane* (food grade) (conforming to IS : 3470-1966)†.

3.2.1 The solvent residues in the finished product shall not exceed the limits compatible with good manufacturing practices.

3.3 Particle Size — The material shall be such that it passes completely through 150-micron IS Sieve (*see* IS : 460 (Part II)-1978‡), or as agreed to between the buyer and the supplier.

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*Rules for rounding off numerical values (*revised*).
†Specification for hexane, food grade.
‡Specification for test sieves: Part II Perforated plate test sieves (*second revision*).

3.4 The material shall also comply with the requirements given in Table 1.

TABLE 1 REQUIREMENTS FOR FISH PROTEIN CONCENTRATE

(*Clause* 2.2 and 3.4)

Sl No.	Characteristic	Requirement	Method of Test Ref to Appendix
(1)	(2)	(3)	(4)
i)	Moisture, percent by mass, *Max*	10	A
ii)	Crude protein (N×6·25), on dry basis, percent by mass, *Min*	70	B
iii)	Available lysine, g/100 g of protein, *Min*	6.5	C
iv)	Fat, on dry basis, percent by mass, *Max*	0.75	D
v)	Ash, on dry basis, percent by mass, *Max*	20	E
vi)	Acid-insoluble ash, on dry basis, percent by mass, *Max*	0.5	F
vii)	Fluorine (as F), mg/kg, *Max*	250	G
viii)	Mercury, mg/kg, *Max*	0.5	H
ix)	Lead, mg/kg, *Max*	2.5	J

3.5 Protein Efficiency Ratio (**PER**) — The protein efficiency ratio when determined by the method given in IS : 7481-1974* shall not be less than 2·5.

3.6 Bacteriological Requirements — The material shall also comply with the bacteriological requirements given in Table 2.

TABLE 2 BACTERIOLOGICAL REQUIREMENTS FOR FISH PROTEIN CONCENTRATE

Sl No.	Characteristic	Requirement	Method of Test Ref to Appendix
(1)	(2)	(3)	(4)
i)	Total bacterial plate count, *Max*	15 000/g	IS : 5402-1969*
ii)	*E. coli* and pathogenic organisms including *Salmonella*	Nil	IS : 5887-1976 (Part I and III)†

*Method for standard plate count of bacteria in foodstuffs.

†Methods for detection of bacteria responsible for food poisoning: Part I Isolation and identification of enteropathogenic *Escherichia coli* and the enumeration of *Escherichia coli*: Part III Isolation and identification of *Salmonella* and *Shigella*.

---

*Method for determination of protein efficiency ratio (PER).

4. PACKING AND MARKING

4.1 Packing — The fish protein concentrate (FPC) shall be packed in clean, sound containers made of tinplate, PCRC sheets, cardboard, paper or other material agreed to between the purchaser and vendor in such a way as to protect it from spillage, contamination, migration of moisture or air from atmosphere and seepage of fat into the material through the packing material. When packed in flexible material, the packaging material should be capable of withstanding rough handling during transportation. The FPC shall not come in direct contact with packaging materials other than grease-proof or sulphate paper, cellulose paper or any other non-toxic packing material which may be covered into moisture-proof laminate or coated paper. When packed in metallic containers, the container shall be airtight and completely filled so as to have minimum air, or the space shall be filled with inert gas, or the contents held in vacuum.

4.2 Marking — The following shall be marked clearly and legibly on the container.

a) Name of the material,

b) Name and address of the manufacturer,

c) Batch/Code number,

d) Minimum net mass and gross mass,

e) Date of manufacture, and

f) Any other requirements under the Standards of Weights and Measures (Packaged Commodities) Rules, 1977.

4.2.1 Each container may also be marked with the ISI Certification Mark.

NOTE — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard, under a well defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

5. SAMPLING

5.1 Representative samples of the material for the tests and criteria for conformity shall be drawn according to the methods prescribed in IS : 5315-1978*.

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*Methods of sampling for milled cereals and pulses products (*first revision*).

6. TESTS

6.1 Tests shall be carried out as prescribed in **3.5** and Col 4 of Tables 1 and 2.

6.2 Quality of Reagents — Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1977*) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the test results.

APPENDIX A

[*Table* 1, *item* (i)]

DETERMINATION OF MOISTURE

A-1. PROCEDURE

A-1.1 Weigh accurately about 5 g of the material in an aluminium dish having a diameter of at least 50 mm and a depth not exceeding 40 mm, previously dried in an oven and weighed. Shake the dish until the contents are evenly distributed. With the cover removed, place the dish to dry in an air-oven maintained at 100±2°C. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing until the difference in mass between two successive weighings is less than one milligram. Record the lowest mass.

A-2. CALCULATION

A-2.1 Moisture, percent by mass $= \dfrac{100 (M_1 - M_2)}{M_1 - M}$

where

M_1 = mass, in g, of the dish with the material before drying;

M_2 = mass, in g, of the dish with the material after drying; and

M = mass, in g, of the empty dish.

---

*Specification for water for general laboratory use (*second revision*).

APPENDIX B

[*Table* 1, *item* (ii)]

DETERMINATION OF CRUDE PROTEIN

B-1. METHOD

B-1.1 The percentage of crude protein is ascertained by multiplying the percentage of nitrogen other than ammoniacal nitrogen by 6.25. The quantity of ammoniacal nitrogen (*see* **B-5.2**) is separately determined and deducted from the total nitrogen (*see* **B-5.1**).

B-2. APPARATUS

B-2.1 Kjeldahl Flask — 500-ml capacity.

B-2.2 Distillation Assembly — The assembly consists of a round bottom flask of 1000-ml capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube. The other end of the bulb tube is connected to a condenser which is attached by means of a rubber tube to a dip tube which dips into a known quantity of standard sulphuric acid contained in a conical flask of 500-ml capacity to which 3 to 4 drops of methyl red indicator solution have been added.

B-3. REAGENTS

B-3.1 Potassium Sulphate or Anhydrous Sodium Sulphate

B-3.2 Copper Sulphate

B-3.3 Concentrated Sulphuric Acid — sp gr 1·84 (*see* IS : 266-1961)*.

B-3.4 Sodium Hydroxide Solution — Dissolve about 450 g of sodium hydroxide in 1000 ml of water.

B-3.5 Standard Sulphuric Acid — 0·1 N

B-3.6 Standard Sodium Hydroxide Solution — 0·1 N

B-3.7 Methyl Red Indicator Solution — Dissolve 0·15 g of methyl red indicator in 500 ml of water (*see* IS : 2263-1962)†.

B-3.8 Magnesium Oxide (Carbonate Free) — Freshly ignited.

---

*Specification for sulphuric acid (*revised*).

†Methods of preparation of indicator solutions for volumetric analysis.

B-4. PROCEDURE

B-4.1 For Total Nitrogen — Transfer carefully about 0·2 g of the accurately weighed, oven dried material, to the Kjeldahl flask. Add about 10 g of potassium sulphate or anhydrous sodium sulphate, about 0·5 g of copper sulphate and 25 ml or more, if necessary, of concentrated sulphuric acid. Place the flask in an inclined position, and heat to the boiling point of the acid until frothing ceases. Increase heat until the acid boils vigorously and digest for about two hours after the mixture is clear. Cool the contents of the flask. Transfer quantitatively to the round bottom flask, with water, the total quantity of water used being about 200 ml. Add a few pieces of pumice stone to prevent bumping. Add sufficient sodium hydroxide solution to make the solution alkaline, carefully down the side of the flask so that it does not mix at once with the acid solution but forms a layer below the acid layer. Assemble the apparatus taking care that the tip of the dip tube extends below the surface of the standard sulphuric acid (25 ml) in the receiver. Mix the contents of the flask by shaking and distil until all ammonia has passed over into the standard sulphuric acid. Titrate the excess of acid with the standard sodium hydroxide solution using methyl red indicator.

B-4.1.1 Carry out a blank determination using all reagents in the same quantities but without the material.

B-4.2 For Ammoniacal Nitrogen — Weigh accurately 2 to 4 g of the dried material. Shake it well with water and filter. Wash the residue thoroughly with water. Transfer the filtrate to the distillation flask and dilute to about 200 ml with water. Add about 5 g of magnesium oxide. Connect the flask to the condenser by means of the connecting bulb tube and distil about 100 ml of liquid into the receiver containing standard sulphuric acid and methyl red indicator solution. Titrate the contents of the receiver with the standard sodium hydroxide solution.

B-4.2.1 Carry out a blank determination using all reagents in the same quantities but without the material.

B-5. CALCULATION

B-5.1 Total nitrogen, percent by mass $= X = \dfrac{1·4\ (V - v)\ N}{M}$

where

V = volume, in ml, of the standard sodium hydroxide solution used to neutralize the acid in blank determination (**B-4.1.1**);

v = volume, in ml, of the standard sodium hydroxide solution used to neutralize the excess acid in the test with the material (**B-4.1**);

N = normality of the standard sodium hydroxide solution; and

M = mass, in g, of the dried material taken for the test (**B-4.1**).

B-5.2 Ammoniacal nitrogen,

$$\text{percent by mass} = Y = \frac{1.4\,(V-v)\,N}{M}$$

where

V = volume, in ml, of the standard sodium hydroxide solution used to neutralize the acid in blank determination (**B-4.2.1**);

v = volume, in ml, of the standard sodium hydroxide solution used to neutralize the excess acid in the test with the material (**B-4.2**);

N = normality of the standard sodium hydroxide solution; and

M = mass, in g, of the dried material taken for the test (**B-4.2**).

B-5.3 Crude protein, on moisture-free basis,

$$\text{percent by mass} = 6.25\,(X - Y)$$

where

X = percent by mass of total nitrogen (B-5.1); and

Y = percent by mass of ammoniacal nitrogen (B-5.2).

APPENDIX C

[*Table* 1, *item* (iii)]

DETERMINATION OF AVAILABLE LYSINE

C-1. PRINCIPLE

C-1.1 The method depends upon the reaction of fluorodinitrobenzene (FDNB) with the end-NH_2 groups of the lysine units in intact food proteins, and colourimetric estimation of the DNP-lysine obtained by subsequent acid hydrolysis.

C-2. APPARATUS

C-2.1 Photo-electric Absorptionmeter — Set at 435 nm or fitted with a blue filter having a maximum transmission close to 435 nm.

C-2.2 Round-Bottom Flask Fitted with Reflux Condenser — 100 ml capacity.

C-2.3 Water Bath — Maintained at 100°C.

C-2.4 Volumetric Flasks — 100-ml and 200-ml capacity.

C-2.5 Burette — 25-ml capacity, graduated at 0·05 ml intervals.

C-2.6 Conical Flask

C-2.7 Pasteur Pipette

C-3. REAGENTS

C-3.1 Sodium Bicarbonate Solution — 8 percent (*m*/*v*).

C-3.2 Fluorodinitrobenzene (FDNB) Solution — Prepare fresh daily and for every sample use approximately 0·3 ml of fluorodinitrobenzene (pipetted after warming the bottle) dissolved in approximately 12 ml of ethanol.

C-3.3 Standard Hydrochloric Acid — 1 and 8·1 N.

C-3.4 Sodium Hydroxide Solution — 2 N.

C-3.5 Phenolphthalein Solution — 1 percent in 70 percent ethanol.

C-3.6 Buffer Solution — 19 parts of 8 percent sodium bicarbonate and one part of 8 percent sodium carbonate, adjusted suitably to 8·5 *p*H with the addition of a little acid or alkali.

C-3.7 Methyl Chloroformate

C-3.8 Di-ethyl Ether — free from peroxide.

C-3.9 Standard DNP-Lysine Hydrochloride Monohydrate — Dissolve, accurately weighed, 24 mg of DNP-lysine hydrochloride monohydrate in 500 ml of 1 N hydrochloric acid solution. Two millilitres of this solution will contain 39·85 μg of available lysine.

C-4. PROCEDURE

C-4.1 The procedure shall be followed in duplicate and away from direct or strongly reflected sunlight.

NOTE — Routine pipetting of ether and fluorodinitrobenzene by mouth is not recommended. An automatic sucker, such as the pro-pipette is recommended and the same sucker may be used for several pipettes.

C-4.1 Weigh accurately about 0·75 g of the material into a 100 ml round bottom flask and shake gently with 8 ml of sodium bicarbonate solution for 10 minutes. Add fluorodinitrobenzene solution. Shake gently but continuously for 2 hours. Evaporate off the ethanol on a boiling water-bath. Add 24 ml of 8·1 N hydrochloric acid and reflux gently for 16 hours. (Some yellow crystals may appear on the condenser. These are not DNP-lysine and need not be recovered). Cool suitably for easy filtration. Filter the contents with water washings and makeup the volume of the filtrate to 200 ml.

C-4.2 Transfer accurately 30 ml of the aliquot of the filtrate to a 50-ml flask and make up to volume.

C-4.3 Transfer 2 ml of the diluted filtrate to a glass stoppered tube. Extract with 5 ml portions of diethyl ether each time until the final ether fraction is colourless, sucking off the ether layer with the pasteur pipette. Remove dissolved ether by standing the tube in hot water.

C-4.4 Make up the volume to 10 ml with 1 N hydrochloric acid and read the extinction in a 1 cm cell with the photo-electric absorption meter at 435 nm.

C-4.5 For the blank, take a second aliquot of 2 ml diluted filtrate (*see* **C-4.2**) in a tube and extract with ether as for the first, also take a third aliquot of 2 ml in a small conical flask for a dummy titration. Dilute and add phenolphthalein indicator and titrate with 2 N sodium hydroxide taken in a burette. Note the volume of sodium hydroxide needed and discard the flask. Add the same volume to the second tube and then 2 ml of buffer solution of *p*H 8·5 (continue without pause from this stage onwards as DNP-compounds are less stable at this *p*H).

C-4.6 Add 0·05 ml of methyl chloroformate to the second tube. Shake and wait for 5 to 10 minutes. Then add 0·75 ml of concentrated hydrochloric acid continuously to avoid excessive effervescence. Extract again with two 5 ml portions of diethyl ether. Evaporate off any residual ether and make up to 10 ml with water. Read the extinction of the tube.

C-4.7 Carry out the experiment through **C-4.3** to **C-4.6** with the standard DNP-lysine solution.

C-5. CALCULATION

C-5.1 Available lysine, g/100 g of crude protein

$$= \frac{X_1 - X_2}{S_1 - S_2} \times \frac{39 \cdot 85}{1000\,000} \times \frac{50}{2} \times \frac{200}{30} \times \frac{100}{W\,(100 - M)} \times \frac{100}{P}$$

where

X_1 = absorptionmeter reading with unknown;

X_2 = blank absorptionmeter reading with unknown;

S_1 = Absorption meter reading with standard;

S_2 = blank absorptionmeter reading with standard;

W = mass, in g, of the material;

M = moisture, percent by mass, in the material; and

P = crude protein (on dry basis) percent by mass, in the material.

APPENDIX D

[*Table* 1, *item* (iv)]

DETERMINATION OF CRUDE FAT

D-1. REAGENT

D-1.1 Petroleum Ether — of boiling point range 40 to 60°C.

D-2. PROCEDURE

D-2.1 Weigh accurately about 5 g of the material, duly dried as described in **A-1.1**. Extract the material with petroleum ether, for 8 hours, in a soxhlet or other suitable extractor. Dry the extract on a steam-bath for 30 minutes, cool in a desiccator and weigh. Repeat the process of drying, cooling and weighing at 30 minute intervals until the difference between two successive weighings is less than one milligram. Note the lowest mass.

D-3. CALCULATION

D-3.1 Crude fat, on dry basis, percent by mass $= \frac{100\ (M_1 - M_2)}{M}$

where

M_1 = mass, in g, of the extraction flask with the dried ether extract;

M_2 = mass, in g, of the extraction flask; and

M = mass, in g, of the dried material taken for the test.

APPENDIX E

[*Table* 1, *item* (v)]

DETERMINATION OF TOTAL ASH

E-1. PROCEDURE

E-1.1 Transfer about 2 g of the dried material (**A-1.1**) to a tared silica dish and weigh. Incinerate the material at low heat not exceeding dull red in the beginning and later at 600 ± 20°C until the ash is free from all carbonaceous material and is white or greyish white. Cool in a desiccator. Weigh if necessary, and repeat the process of igniting, cooling and weighing until the difference between two successive weighings is less than one milligram. Note the lowest mass.

NOTE — Preserve the dish containing this ash for the determination of acid-insoluble ash.

E-2. CALCULATION

E-2.1 Total ash, on dry basis, percent by mass $= \frac{100\ (M_2 - M)}{M_1 - M}$

where

M_2 = mass, in g, of the dish with ash;

M = mass, in g, of the empty dish; and

M_1 = mass, in g, of the dried material taken for the test.

APPENDIX F

[*Table* 1, *item* (vi)]

DETERMINATION OF ACID-INSOLUBLE ASH

F-1. REAGENT

F-1.1 Hydrochloric Acid — Approximately 5 N, prepared from concentrated hydrochloric acid conforming to analytical reagent grade of IS : 265-1962*.

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*Specification for hydrochloric acid (*revised*).

F-2. PROCEDURE

F-2.1 To the ash contained in the silica dish (**E-1.1**), add 25 ml of hydrochloric acid. Cover with a watch glass and heat on a water bath for 10 minutes. Allow to cool and filter the contents of the dish through a Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from acid. Return the filter paper and the residue to the dish. Keep it in an electric air-oven maintained at 135±2°C for about three hours. Ignite in a muffle furnace at 600±20°C for three hours. Cool the dish in a desiccator and weigh. Repeat the process of igniting in the muffle furnace, cooling and weighing at half-hour intervals until the difference between the two successive weighings is less than one milligram. Note the lowest mass.

F-3. CALCULATION

F-3.1 Acid insoluble ash, on dry basis, percent by mass $= \frac{100\ (M_2 - M)}{M_1 - M}$

where

M_2 = mass, in g, of the dish with the acid-insoluble ash;

M = mass, in g, of the empty dish; and

M_1 = mass, in g, of the dish with the dried material taken for the determination of acid-insoluble ash.

APPENDIX G

[*Table* 1, *item* (vii)]

DETERMINATION OF FLUORINE

G-0. PRINCIPLE

G-0.1 The sample is ashed with $Ca(OH)_2$ as a fluorine fixative, and fluorine is isolated by distillation from $HClO_4$ and estimated in the distillate by thorium nitrate back-titration.

G-1. APPARATUS

G-1.1 Steam distillation apparatus — (*see* Fig. 1)

G-1.2 Nessler Tubes — 100 ml capacity.

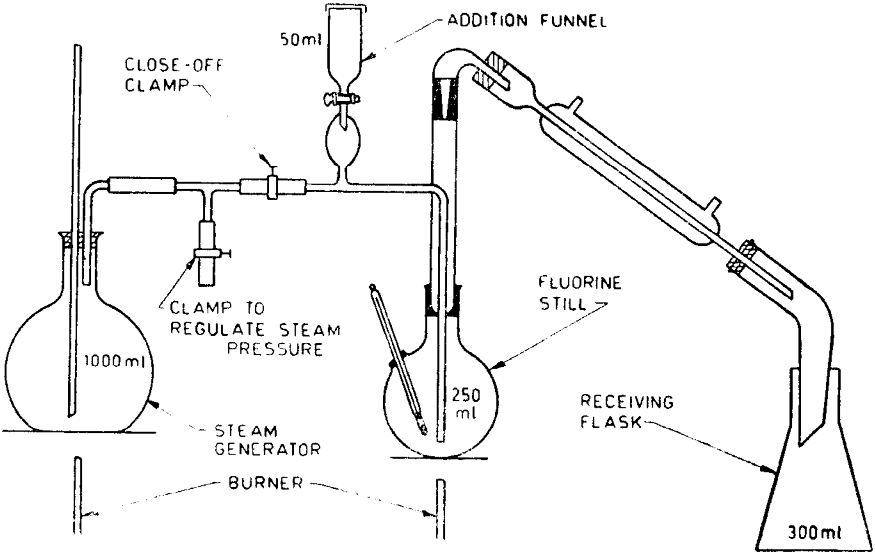

FIG. 1 STEAM DISTILLATION APPARATUS FOR THE ISOLATION OF FLOURINE BY THE MODIFIED METHOD

G-1.3 Platinum crucibles

G-1.4 Heating Lamp

G-1.5 Muffle Furnace

G-1.6 Vacuum Desiccator containing Silica Gel

G-2. REAGENTS

G-2.1 Calcium Hydroxide — 1 mole $Ca(OH)_2$/litre.

G-2.2 Thorium Nitrate Solution — 2.7×10^{-4} *M*.

G-2.3 Silver Perchlorate Solution — 50 g/100 ml.

G-2.4 Perchloric Acid — 60 percent.

G-2.5 Sodium Hydroxide — 0·05 N.

G-2.6 p-Nitrophenol — 0·5 percent alcoholic solution.

G-2.7 Buffer — Mix 400 ml, 0·2 N HCl and 4·4 g $NH_2OH.HCl$ and dilute to 2 litres with water.

G-2.8 Indicator — 0·01 percent sodium alizarin sulphonate.

G-3. PREPARATION OF SAMPLE

G-3.1 Weigh 0·5 – 1·0 g sample directly into platinum crucibles. Add glycerine to hydrophobic samples to make them wettable. Fix fluorine by adding 20 ml $Ca(OH)_2$ suspension. Place the mixture under a heating lamp and bring to just dryness, and ash for about 16 hours in a muffle furnace at 550°C. Store ashed sample in a vacuum desiccator over silica gel.

G-4. ISOLATION OF FLUORINE

G-4.1 Add excess silver perchlorate (usually 2 g) to still pot to supress formation of HCl. Also add 10-15 small (1 mm) borosilicate glass beads and about 0·05 g finely-ground silica sand. Use a total of about 40 ml perchloric acid for distillation. Cover ashed sample in platinum crucible with watch glass and dissolve sample in minimum of acid. Introduce sample and remainder of acid into still pot immediately, through addition funnel. Thoroughly rinse all apparatus used to transfer sample and add rinse to still with enough water to make total volume about 150 ml. Distil at 125 ± 5°C at 3-4 ml/min until about 300 ml distillate is collected. Keep distillate just alkaline to *p*-nitrophenol by drop-wise additions of 0·05 N sodium hydroxide. Generally not more than 2 ml of sodium hydroxide solution is necessary.

G-5. TITRATION OF FLUORIDE

G-5.1 Prepare calibration curve of 10-50 μg fluoride standards every time titrations are performed. Determine fluorine by direct titration with the thorium nitrate solution in matched, 100-ml Nessler tubes, using fluorescent light for illumination. Add 10 ml HCl - hydroxylamine buffer and 1 ml indicator solution to each titration tube. After all tubes are thus prepared, add just enough thorium nitrate solution to one tube to produce first pink colour (as viewed through depth of tube) and fill to the 100-ml mark with water. Volume of thorium nitrate solution added is the titration blank. Use this tube as colour standard to compare all subsequent titration end-points. Determine end-point only when tubes are filled to the 100-ml mark.

G-5.2 Neutralize excess NaOH in distillate to *p*-nitrophenol end-point (colourless) with buffer solution and make to 300 ml with water. Titrate suitable aliquots (25 to 75 ml) with thorium nitrate against colour standard tubes. Correct for titration blank. Determine amount of fluorine in aliquot from calibration curve. Correct fluoride content for distillation blank, which should be determined periodically to ensure accurate results.

G-6. CALCULATION

G-6.1 Fluoride, mg/kg $= \frac{M}{W}$

where

M = μg of fluorine in sample, and
W = mass of sample in g.

APPENDIX H

[*Table* 1, *item* (viii)]

DETERMINATION OF MERCURY

H-1. PRINCIPLE

H-1.1 Sample is digested with nitric acid (HNO_3) and sulphuric acid (H_2SO_4) under reflux in a special apparatus. Mercury is isolated by dithizone extraction, copper is removed and mercury is estimated by photometric measurement of mercury dithizonate.

H-2. APPARATUS

H-2.1 Special Digestion Apparatus — (*see* Fig. 2). The apparatus is made from pyrex glass with standard taper joints throughout. Unit A is a modified soxhlet extractor, of 5 cm outer diameter and 200 ml capacity, without an inner siphon tube but equipped with a stopcock on the tube leading to digestion flask, D. With the stopcock open, the apparatus is in reflux position; when closed, the unit serves as a trap for condensed water and acids. The top of *A* is attached to a Friedrich's condenser, 35 cm long. The bottom of *A* is attached to the centre neck of a 2-neck standard taper 24/40 round-bottomed 500-ml flask, D. Necks are 3 cm apart to provide clearance. The second neck is used for attaching a 75-ml dropping funnel *B*.

H-3. REAGENTS

H-3.1 Mercury Standard Solutions

H-3.1.1 *Stock Solution* — 1 mg/ml; prepared from dry, recrystallized mercuric chloride ($HgCl_2$) (67·7 mg/50 ml).

H-3.1.2 *Working Solution* — 2 μg/ml is convenient. Prepare from stock solution and store in pyrex bottles. Add hydrochloric acid (HCl) in a proportion of 8 ml/litre to all standards before diluting to final volume.

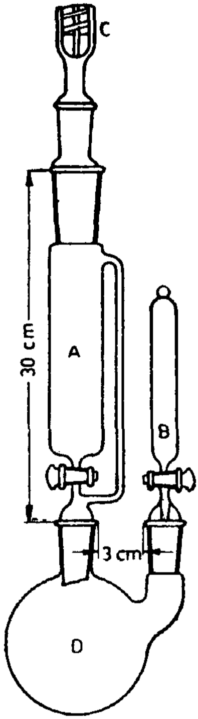

FIG. 2 SPECIAL DIGESTION APPARTUS FOR DETERMINATION OF MERCURY RESIDUES

H-3.2 Chloroform ($CHCl_3$) — Distil from hot water-bath, collecting distillate in absolute alcohol in the proportion of 10 ml alcohol to 1 litre distillate. Intermittently shake receiver during distillation.

H-3.3 Dithizone Solution — Dissolve about 1 g commercial reagent in 50-75 ml $CHCl_3$ and filter if insoluble material remains. Extract in a separator with four 100 ml portions of metal-free (redistilled) NH_4OH (1 : 99) (dithizone passes into the aqueous phase to give an orange solution). Filter aqueous extracts into a large separator through a cotton plug inserted in the stem of the funnel. Acidify slightly with dilute HCl and extract dithizone with two or three 20 ml portions of $CHCl_3$. Combine extracts in separator and wash 2 or 3 times with water. Drain $CHCl_3$ into a beaker and evaporate with gentle heating on a steam bath, avoiding sputtering as the solution goes to dryness. Remove last traces of moisture by heating for 1 hour at about 50° in vacuum. Store dry reagent in the dark in a tightly stoppered bottle. Prepare stock solution in redistilled $CHCl_3$ (100 mg/litre is convenient) and store in a refrigerator. Prepare dilutions as needed.

H-3.4 Sodium Thiosulphate Solution — 1·5 percent prepared daily.

H-3.5 Sodium Hypochlorite Solution (**NaOCl**) — Preferably 5 percent available chlorine reagent. The reagent varies in available chlorine content. Determine the strength. Store in refrigerator when not in use and determine titer monthly. (Certain preparations of hypochlorite intended for household use contain traces of Hg. If these preparations are used, determine blank. Reagent containing more than 0·1 µg Hg/ml should not be used.)

H-3.6 Dilute Acetic Acid — 30 percent (*v/v*).

H-3.7 Hydroxylamine Hydrochloride Solution — ($\mathbf{H_2NOH.HCl}$) — 20 percent (*m/v*). Extract with dilute dithizone until $CHCl_3$ layer remains green, remove excess dithizone with $CHCl_3$ and filter.

H-4. PREPARATION OF SAMPLE

H-4.1 Place weighed sample in digestion flask with 6 glass beads. Add 20 ml HNO_3 to the sample, swirl flask, and let it stand for half an hour in digestion assembly before heating. Add 25 ml water and heat cautiously with small rotating flame until the initial vigorous reaction is over and foaming ceases. (Original reaction must not proceed violently or the evolved nitrogen peroxide (NO_2) will carry vapours of digest mechanically through the condenser and cause loss of Hg). After initial reaction is the complete, apply heat so that digest just refluxes. If the mixture darkens, add HNO_3 dropwise through the funnel as needed. Continue refluxing for half an hour or until the digest does not change consistency, and cool.

H-4.2 Slowly add 20 ml of cold HNO_3-H_2SO_4 mixture (1 : 1). [Use 100 ml of acid mixture for about 5 g (dry mass) of sample]. Heat with a small flame, subsequently adding HNO_3 dropwise as needed to dispel darkening of the digest. Continue heating until the fibrous material is apparently digested. Turn the stopcock of the soxhlet unit to trap water and acids, and continue heating. Let the digest become dark brown (not black) before adding further increments of HNO_3. (Fats and waxes cannot be totally digested by hot acids under reflux. Therefore, no attempt should be made to effect complete digestion in this step). When all except fat and wax is in solution, let digest cool, and cautiously drain water and acids into main digest. Cool and pour two 25-ml portions of water through condenser and intermediate unit. Remove reaction flask, chill under cold water or by surrounding with ice to solidify fats and waxes, and filter off insoluble matter on small plug of glass wool. Rinse reaction flask and filter pad successively with two 10-ml portions of water. Remove soxhlet unit and wash it and flask with hot water to remove insoluble material. Pour hot water through condenser to remove volatile fats and oils. Discard all washings.

H-4.3 Connect flask containing filtered sample solution to assembled apparatus, heat and collect water and acids in trap. Complete digestions, using small additions of HNO_3 as needed. In final stages of digestion, adjust flame until digest reaches incipient boiling (solution simmers) and acid vapours do not rise beyond lower half of condenser. Continue heating 15 minutes after last addition of HNO_3. Digest should now be colourless or pale yeallow. Let digest cool, drain trapped liquids carefully into reaction flask, and add two 50-ml portions of water through condenser. Reflux solution until all NO_2 is expelled from apparatus. Add 5 ml 40 percent *m/v* urea solution and reflux for 15 minutes. Digest should be colourless or pale yeallow.

H-5. ISOLATION OF MERCURY

H-5.1 Titrate 1 ml of prepared sample solution (**H-4.3**) with standard alkali. Add a calculated amount of concentrated ammonia to reduce acidity to 1·0 N; swirl flask during addition of the ammonia to avoid local excess (solution should never be ammoniacal to avoid formation of mercury complexes).

H-5.2 Transfer sample solution to a 500-ml separator. Add 10 ml of 4 mg/litre dithizone and shake vigorously for 1 minute. (If the characteristic green colour of dithizone is visible in the $CHCl_3$ layer, indicating excess of dithizone, the amount of Hg is within 0·5 μg). Let the layers separate and drain $CHCl_3$ layers quickly to a second separator containing 25 ml of 0·1 N HCl and 5 ml $H_2NOH.HCl$ solution. (Small amount of oxidizing material may still be present. On long contact with dithizone solution, xidizing substances may destroy dithizone reagent and prevent extraction of mercury)

H-5.3 Repeat extraction of sample solution with two 5-ml portions of dithizone solution, transferring $CHCl_3$ layer successively to second separator. If first extraction indicates more than 5 μg Hg, add stronger concentrations of dithizone, as indicated by table under **H-7.1**, until after 1 minute of vigorous shaking, $CHCl_3$ layer contains dithizone in marked excess. Drain $CHCl_3$ layer into second separator containing 0·1 N HCl and again extract sample solution with two 10-ml portions of 4 mg/litre dithizone solution, draining each successive extract into second separator.

H-5.4 Shake the contents of second separator vigorously for 1 minute and drain $CHCl_3$ layer into a third separator containing 50 ml of 0·1 N HCl. (Shaking dithizone extract with dilute acid in second separator removes entrained organic matter. With biological materials or those of high protein content, aqueous layer is usually light yellow because of nitrated organic compounds. Small amounts are carried into the third separator where they are destroyed by chlorine). Extract solution in second separator with 1-2 ml $CHCl_3$ and transfer organic layer to third separator.

H-5.5 To the contents of the third separator add 2 ml sodium thiosulphate solution, shake vigorously for 1 minute, let layers separate, drain off $CHCl_3$ as completely as possible, and discard. (Copper if present is removed as dithizonate). Extract again with 1-2 ml $CHCl_3$, drain carefully, and discard. Add 3·5 ml NaOCl reagent (or enough solution of different titer to furnish 175 mg of available chlorine) to decompose mercury thiosulphate complex and oxidize excess thiosulphate, and shake vigorously for 1 minute. Add 5 ml H_2N OH.HCl reagent from pipette, taking care to wet both stopper and neck of separator. Shake vigorously for 1 minute. Hold the mouth of the separator in front of the air vent and blow out any remaining gaseous chlorine. Stopper separator and shake vigorously for 1 minute. (It is imperative that all hypochlorite be reduced. Trace amounts remaining would oxidize dithizone, subsequently added, to a yellow oxidised form which would be measured in a photometer as mercury. Extract the solution with 2-3 ml of $CHCl_3$, drain off the organic layer carefully, and discard. Final aqueous solution should be colourless.

H-6. DETERMINATION

H-6.1 To the third separator, add 3 ml of 30 percent acetic acid and an appropriate volume and concentration of dithizone solution as indicated in the table (*see* **H-7.1**) and proceed with colourimetric determination of mercury as in **H-7**, converting absorbance (*A*) measured at 490 nm to µg mercury from the working curve.

H-7. PREPARATION OF STANDARD CURVE

H-7.1 The following table is useful in preparing the standard curve and for establishing appropriate mercury range in sample solutions when 1-cm cell is used.

Mercury Range µg (1)	*Dithizone concentration* (mg/litre) (2)	*Volume dithizone* (ml) (3)
0 – 10	6	5
0 – 50	10	25
0 – 100	10	40

H-7.2 Prepare a working curve of required range, starting with the blank and extending to final standard of the range, with 4 intermediate increments. Add appropriate amounts of mercury to 50 ml 0·1 N HCl in a separator. Add 5 ml $H_2NOH.HCl$ reagent and 5 ml $CHCl_3$ and shake vigorously for 1 minute. Let the layers separate, drain off $CHCl_3$ and discard, being careful to remove as completely as possible all droplets of $CHCl_3$. Add 3 ml of 30 percent acetic acid and an appropriate volume of dithizone solution, shake vigorously for one minute, and let the layers separate (acetic acid aids in stablizing mercuric dithizonate). Insert cotton plug into the stem of the separator and collect dithizone extract (discarding the first ml) in test tube for transfer to appropriate cell. Make photometer readings at 490 nm (since both dilute dithizone and mercuric dithizonate are somewhat unstable; read immediately). Plot A against μg mercury.

H-8. PRECAUTIONS

H-8.1 The critical step is digestion of the sample, which must be almost complete, otherwise the residual organic matter may combine with mercury and prevent or hinder extraction with dithizone. The oxidising material in digest must also be destroyed otherwise the dithizone reagent is decomposed and mercury is not quantitatively extracted. Because of volatility of mercury components, careful heating of digest during sample preparation is required. Acidity of final sample solution (after partial neutralization with ammonia) before extraction should be about 1 N and not more than 1·2 N. Do not use silicone grease in stopcocks.

APPENDIX J

[*Table* 1, *item* (ix)]

DETERMINATION OF LEAD

J-1. APPARATUS

J-1.1 Digestion Funnel

J-1.2 Separatory Funnel

J-2. REAGENTS

J-2.1 Nitric Acid — 65 percent.

J-2.2 Sulphuric Acid — Sp gr 1·84.

J-2.3 Ammonium Acetate-Citrate Solution — Dissolve 12·5 g of ammonium acetate and 12·5 g of ammonium citrate in water, add concentrated ammonia until the solution is alkaline to thymol blue paper and add water to make it to 100 ml. Purify with 0·002 percent *m/v* solution of dithizone in carbon tetrachloride, and finally shake the solution with carbon tetrachloride to remove excess of dithizone.

J-2.4 Ammonia Solution — 25 percent.

J-2.5 Carbon Tetrachloride

J-2.6 Ammonium Hydroxide — 0·2 N.

J-2.7 Potassium Cyanide — 10 percent.

J-2.8 Hydroxylamine Hydrochloride Solution — 10 percent.

J-2.9 Dithizone Solution — 0·1 percent (*m/v*), purified by the following procedure:

Dissolve the dithizone in chloroform and treat it with ammonia. Add mineral acid. Precipitate shall be pure dithizone. The aqueous ammoniacal solution obtained from a chloroform solution of dithizone should be colourless; otherwise further purification as described above should be carried out.

J-2.10 Buffer *p*H — Add 11·90 ml of 0·2 M hydrochloric acid and 88·10 ml of 0·2 M potassium chloride in a 200 ml volumetric flask and add water to make up the volume.

J-3. PROCEDURE

J-3.0 The limit test described for lead is designed to show that a sample contains more than either 10 mg/kg or 20 mg/kg of lead. The sample is digested with nitric and sulphuric acids, and a clear solution of the digest is prepared.

J-3.1 Digestion — Weigh 4·0 g of sample. Place with 5 ml of water, 5 ml of 65 percent nitric acid and 5 ml of sulphuric acid in a digestion flask. Warm slightly. If foaming becomes excessive, add a little water. Evaporate the mixture. Maintain strongly oxidizing conditions in the flask during digestion by adding cautiously small quantities of nitric acid whenever the mixture begins to turn brown or dark. Continue digestion until organic matter is destroyed and sulphur trioxide fumes are copiously evolved. The final solution should be colourless or at the most a slight straw colour.

J-3.1.1 To remove nitrosylsulphuric acid, after partial cooling transfer the residue to a dish, rinse with 25 ml of water, evaporate and heat again to fuming point. Add 25 ml of water and evaporate and heat again.

J-3.2 Solution of Digest — Allow to cool, add 20 ml of ammonium acetate-citrate solution, and allow to cool again. Neutralize to about *p*H 7 with 25 percent ammonia and boil if necessary to dissolve calcium sulphate. Cool the clear solution. Shake the solution with 10 ml of carbon tetrachloride and discard the lower layer.

J-3.3 Test with Dithizone — To a 100-ml separatory funnel add 10 ml of 0·2 N ammonium hydroxide, 2 ml of 10 percent potassium cyanide, 2 ml of 10 percent hydroxylamine hydrochloride solution and 2 ml of 20 mg per litre solution of dithizone in carbon tetrachloride. Shake the separatory funnel for a few minutes and discard the carbon tetrachloride layer. Add 2 ml of carbon tetrachloride, shake and discard again the carbon tetrachloride layer. Add the solution of the digest to the separatory funnel. Adjust the *p*H of the solution to about 10 (thymol blue paper) with ammonia. Again add 5 ml of dithizone solution in carbon tetrachloride and shake vigorously for a few minutes. The carbon tetrachloride layer becomes red according to the amount of lead present.

J-3.4 Treat simultaneously a standard solution containing 10 μg of lead in the same manner as the solution of the digest in **J-3.2**. Evaluate the quantity of lead in the digest by comparing the colours of the carbon tetrachloride layers.

J-3.4.1 Ensure that the red colour is due to lead by shaking the carbon tetrachloride layer obtained from the digest solution with 10 ml of a buffer *p*H2. The red colour will turn green if it is due to lead.

APPENDIX K

(*Clause* 0.4)

METHOD OF PREPARATION OF FISH PROTEIN CONCENTRATE

K-1. RAW MATERIAL

K-1.1 Fish protein concentrate can be prepared from a single species or a mixture of trash fish comprising jew fish (*Pseudorciaena* sp), silver belly (*Leiognathus* sp), malabar sole (*Cyanolossus* sp), *dhoma*, etc.

K-2. PREPARATION OF PRESSED CAKE

K-2.1 The raw material is washed to remove extraneous matter and minced without dressing. The minced mass is suspended in water in the ratio of 1 : 1 by mass and glacial acetic acid is added to the extent of 0·5 percent (*m*/*v*) on the basis of the mass of minced muscle. The material is cooked in an open-jacketted kettle at 70-80°C with constant stirring for about 30 minutes. The water is removed by decantation and the mass is pressed in a hydraulic press. The pressed cake goes for solvent extraction.

K-3. SOLVENT EXTRACTION

K-3.1 The wet cake is charged into the extraction cell having heating, stirring and reflux arrangements. The first extraction is carried out using ethyl alcohol in the ratio of two parts by volume of alcohol to one part by mass of cake and kept at boiling temperature for half an hour under constant agitation. The three subsequent extractions, after draining out solvent alcohol are carried out in a similar way using an azeotropic mixture of haxane and ethyl alcohol (bp 58·7°C) at its boiling point, adding the azeotrope to the cake in the ratio of 1 : 5 (*v*/*v*). Other solvents (food grade) such as iso-propanol can also be used to remove fat and odour-bearing compounds from the fish cake.

K-3.1.1 Extraction should continue till the final oil content of the cake is reduced to 0·5 percent or less on dry mass basis. After the final extraction, residual solvent from the mass is removed by centrifugation and subsequent steam stripping and vacuum drying to remove solvent as completely as possible. The dried matter is reduced to the desired particle size by means of a pulveriser. The spent solvent can be recovered by evaporation and condensation and re-used in the process.

(*Continued from page* 2)

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INDIAN STANDARDS ON NUTRITIOUS FOODS

IS:

3137-1974	High-protein mixes for use as food supplement (*first revision*)
4684-1975	Edible groundnut flour (expeller pressed) (*first revision*)
4874-1968	Edible cottonseed flour (expeller pressed)
4875-1975	Edible groundnut flour (solvent extracted) (*first revision*)
4876-1968	Edible cottonseed flour (solvent extracted)
6108-1971	Edible sesame flour (solvent extracted)
6109-1971	Edible sesame flour (expeller pressed)
7021-1973	Protein-rich food supplements for infants and pre-school children
7481-1974	Method for determination of protein efficiency ratio (PER)
7482-1974	Protein-based beverages
7487-1974	Protein-rich biscuits
7835-1975	Edible medium-fat soya flour
7836-1975	Edible low-fat soya flour
7837-1975	Edible full-fat soya flour
8211-1976	Edible soya protein isolate
8212-1976	Edible groundnut protein isolate
8220-1976	Protein rich concentrated nutrient supplementary foods
8222-1976	Edible leaf protein concentrate
8664-1977	Edible coconut flour (expeller pressed)
8665-1977	Protein fortified bread
8676-1977	Edible coconut flour (solvent extracted)
8677-1977	Edible sunflower seed flour (solvent extracted)
8678-1977	Vegetable protein-based yoghurt (vegetable curds)
9037-1979	Peanut butter
9038-1979	Reconstitutable protein beverage powder
9039-1979	Edible sunflower seed grits
9071 (Part I)-1979	Code of practice for control of aflatoxin in groundnuts: Part I Harvesting transport and storage of groundnuts
9071 (Part II)-1979	Code of practice for control of aflatoxin in groundnuts: Part II Plant storage and processing flour and oil
9095-1979	Protein chewy candy
9216-1979	Glossary of common terms relating to nutrition and nutritious foods
9487-1980	'Ready-to-eat' protein-rich-extruded foods
9488-1979	Edible coconut protein concentrates
10038-1981	Textured plant protein foods prepared by extrusion cooking
10059-1981	Edible fish powder